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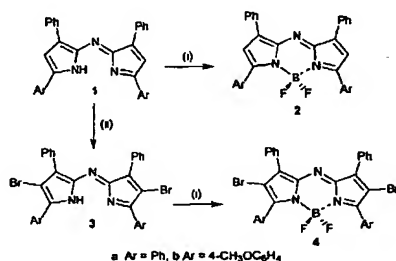
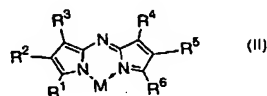
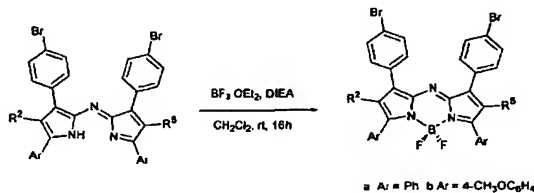
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[Continued on next page]

(54) Title: COMPOUNDS USEFUL AS PHOTODYNAMIC

ITS

## Reaction Scheme

(i) BF<sub>3</sub>·OEt<sub>2</sub>, DIEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 16 h;(ii) Br<sub>2</sub>, toluene, rt, 2 h.

The present invention relates to compounds of the formula (I), or a salt, metal complex or hydrate or other solvate thereof, wherein: M is a chelating agent; R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> are independently selected from the group consisting of: H; a substituted or unsubstituted, saturated or unsaturated, cyclic, moiety; a substituted or unsubstituted, saturated or unsaturated, heterocyclic moiety; or a substituted or unsubstituted, saturated or unsaturated, straight or branched chain alkyl or acyl moiety; and R<sup>2</sup> and R<sup>5</sup> may also be independently a heavy atom or a water-solubilizing group. The present invention also relates to use of these compounds in the therapy *in vivo* or *in vitro* of a photosensitive target biological cell by irradiation, as well as methods of treating a photosensitive target biological cell *in vivo* or *in vitro*. Finally, the present invention relates to pharmaceutical compositions, comprising these compounds, in association with a pharmaceutically acceptable diluent or carrier.



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### Compounds useful as Photodynamic Therapeutic Agents

The present invention relates to compounds useful as photodynamic therapeutic agents and to pharmaceutical compositions containing said compounds. The present invention also relates to methods of photodynamic therapy, by  
5 administration of the said compounds.

Photodynamic therapy (PDT) is a non-invasive technique for the treatment of a variety of solid tumour types by administering a photosensitising compound,  
10 followed by illumination of the tumour with light of a wavelength absorbed by the compound, for example visible or near-visible light. The photosensitising compound is administered first to optimise uptake of the photosensitising compound by the tissue to be treated. A typical time lag between administration of the photosensitising compound and subsequent illumination of the tissue would  
15 be 24 – 48 hours. PDT also has application in certain non-neoplastic diseases including age-related macular degeneration, coronary heart disease and periodontal diseases caused by overgrowth of pathogenic microflora around the teeth. The therapeutic strategy involves contacting a photosensitising compound of low dark toxicity with a target area/tissue, which target area/tissue is in the  
20 body for *in vivo* therapies. The photosensitising compound accumulates preferentially to some extent within the target area/tissue to be treated, e.g., within a tumour. The target area/tissue is then irradiated with low energy light through the body's therapeutic window, i.e. beyond the absorbance of body tissue, (650-850 nm), resulting in excitation of the photosensitising compound. All other  
25 things being equal, the longer the wavelength of the illuminating light within the therapeutic window, the greater the tissue penetration of light and, therefore, the greater the ability to treat deep seated tissues such as deep seated tumours. The light-activated photosensitising compound can then transfer its excited state energy to surrounding biological tissue through molecular oxygen, resulting in  
30 oxidative cellular damage leading to cell death *via* apoptosis and/or necrosis. After light treatment, the photosensitiser is allowed to clear from the body. PDT

can be viewed as a highly selective form of tissue treatment, provided that the photosensitiser is non-toxic in the absence of light (i.e. has a low dark toxicity), so that only the irradiated areas are affected.

- 5 Most known PDT compounds investigated to date are based on cyclic-tetrapyrrole macrocycles, from which it can be difficult to generate a range of sequentially modified derivatives (M. Wainwright, *Chem. Soc. Rev.*, 1996, 351).

At the present time, Photofrin®, a haematoporphyrin derivative, is the most  
10 commonly used clinically available PDT agent. It has been approved for use in the United States, Japan and Europe for the treatment of oesophageal, lung, stomach, and cervical cancers (R. Bonnett, *Chem. Soc. Rev.*, 1995, 24, 19 and T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, and Q. Peng, *J. Natl. Cancer Inst.* 1998, 90, 889). Although it is the most  
15 extensively used anti-cancer PDT agent, it is widely recognised that it is far from being an ideal drug for use in PDT (I.J. MacDonald and T.J. Dougherty, *J. Porphyrins Phthalocyanines*, 2001, 5, 105).

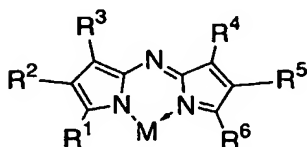
Other more recently approved agents are Foscan and Levulan, both of which are  
20 porphyrin derivatives.

Despite its achievements to date, PDT is still in its developmental stages, with a marked need to develop improved photosensitising compounds with better efficacy and side effect profiles. In order to further advance this novel form of  
25 treatment, it has become apparent that the development of new PDT compounds, together with a more thorough and integrated understanding of the multitude of targets/actions so far ascribed to PDT agents, is needed.

The present invention alleviates the problems of the prior art by providing  
30 synthesis, photophysical properties and *in vitro* cellular uptake evaluation of a new class of potential PDT agent, derived from azadipyrromethenes whose

tetraaryl derivatives 1 which were first reported in 1940's but which, since then, have remained relatively unstudied (M.A.T. Rogers, *J. Chem. Soc.*, 1943, 596 and E.B. Knott, *J. Chem. Soc.*, 1947, 1196).

- 5 A first aspect of the present invention, therefore, provides a compound of the formula



- 10 in which M is a chelating agent; R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> can each, independently, be H; a substituted or unsubstituted, saturated or unsaturated, cyclic, preferably aryl, moiety; a substituted or unsubstituted, saturated or unsaturated, heterocyclic, preferably heteroaryl, moiety; or a substituted or unsubstituted, saturated or unsaturated, straight or branched chain alkyl or acyl moiety and R<sup>2</sup> and R<sup>5</sup> can each, in addition and independently, be a heavy atom, preferably a halogen selected from At, I, Br or Cl, of which I or Br are most preferred, or a water-solubilizing group. The present invention also provides salts, metal complexes or hydrates or other solvates particularly with lower, e.g., C<sub>1</sub> - C<sub>4</sub>, aliphatic alcohols of the aforementioned compounds.

- 20 R<sup>1</sup> and R<sup>6</sup> (which may be the same or different, the same being preferred) are at the α-pyrrole positions; R<sup>2</sup> and R<sup>5</sup> (which may be the same or different, the same being preferred) are at the β-pyrrole positions; and R<sup>3</sup> and R<sup>4</sup> (which may be the same or different, the same being preferred) are at the γ-pyrrole positions, all with respect to the N atom of each pyrrole ring.
- 25

Preferably, M is BX<sub>2</sub>, in which each X is, independently, a halide. Most preferably, each halide is a fluoride. Alternatively, M is a metal selected, preferably, from Zn, Al, Si, Mg, Lu and Sn.

As used herein, the term "heavy atom" is intended to embrace atoms with an atomic weight greater than 15, preferably greater than 30, more preferably greater than 35. Selenium is another example of a heavy atom.

5

As used herein, the term "cyclic" is intended to embrace substituted or unsubstituted, saturated or unsaturated, moieties containing one or more rings. If more than one ring is present, the rings may be fused together. Suitable are substituted or unsubstituted steroids.

10

As used herein, the term "aryl", which is included within the scope of "cyclic", is intended to embrace substituted or unsubstituted, unsaturated, monocyclic or polycyclic (fused or separate) aromatic hydrocarbon moieties. Preferred monocyclic aromatic moieties include phenyl, substituted phenyl moieties including, but not limited to, tolyl, xylyl, mesityl, cumenyl (isopropyl phenyl) and substituted phenylene derivatives including, but not limited to, benzyl, benzhydryl, cinnamyl, phenylethyl, styryl and trityl. Preferred fused polycyclic moieties include substituted and unsubstituted naphthalene and anthracene moieties.

20

As used herein, the term "heterocyclic" is intended to embrace substituted or unsubstituted, saturated or unsaturated, monocyclic or polycyclic (fused or separate) heterocyclic moieties. Suitable non-aromatic moieties are substituted or unsubstituted piperidine, dioxane, piperazine and pyrrolidine moieties.

25

As used herein, the term "heteroaryl", which is included within the scope of "heterocyclic", is intended to embrace substituted or unsubstituted, unsaturated, monocyclic or polycyclic (fused or separate) aromatic heterocyclic moieties. Preferred are substituted or unsubstituted pyridine, pyridazine, pyrimidine, pyrazine, purine, furan, pyrrole, benzofuran, indole and thiophene moieties.

30

As used herein, the term "aromatic" is intended to embrace a fully unsaturated, substituted or unsubstituted, cyclic moiety.

As used herein, the term "alkyl" is intended to embrace substituted or unsubstituted, straight or branched chain, saturated or unsaturated C<sub>1-25</sub> alkyl, alkenyl or alkynyl moieties. Preferred are alkyl moieties such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl, isopentyl, hexyl, methylpentyl, isohexyl, heptyl, isoheptyl, octyl, isooctyl, nonyl, isononyl, decyl, isodecyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, icosyl, henicosyl, docosyl, tricosyl, tetracosyl and pentacosyl, all of which may be further substituted. Preferred alkenyl and alkynyl moieties include vinyl, ethynyl, allyl, isopropenyl, propynyl, butenyl, butynyl, pentenyl, pentynyl, hexenyl, hexynyl, heptenyl, heptynyl, octenyl, octynyl, nonenyl, nonynyl, decenyl, decynyl, undecenyl, undecynyl, dodecenyl, dodecynyl, tridecenyl, tridecynyl, tetradecenyl, tetradecynyl, pentadecenyl, pentadecynyl, hexadecenyl, hexadecynyl, heptadecenyl, heptadecynyl, octadecenyl (oleic or elaidic), octadecynyl, nonadecenyl, nonadecynyl, icosenyl, icosynyl, henicosenyl, henicosynyl, docosenyl, docosynyl, tricosenyl, tricosynyl, tetracosenyl, tetracosynyl, pentacosenyl and pentacosynyl, all of which may be further substituted.

As used herein, the term "acyl" is intended to embrace alkyl-CO- moieties.

Advantageously, R<sup>1</sup> and/or R<sup>6</sup> comprise, independently, a cyclic, preferably an aryl, moiety or a heterocyclic, preferably a heteroaryl, moiety (of the latter of which thiophene, furan or pyrrole moieties are preferred). These moieties may be substituted or unsubstituted. Aryl moieties are more preferred, with monocyclic aryl moieties, and in particular phenyl, being most preferred. R<sup>1</sup> and/or R<sup>6</sup> preferably also contain an electron-donating substituent to maximise extinction coefficients and to shift the maximum wavelength of absorption beyond 650nm. Alkoxy (with a C<sub>1-25</sub> alkyl, preferably C<sub>1-10</sub> alkyl, and more preferably C<sub>1-4</sub> alkyl

group), most preferably methoxy, is a preferred electron-donating substituent. Alternatively,  $R^1$  and/or  $R^6$  may comprise, as an electron-donating substituent, a substituted or unsubstituted, saturated or unsaturated, straight or branched chain alkyl moiety (which has from 1 to 25, preferably 1 to 10 and more preferably 1 to 4, carbon atoms).

Advantageously,  $R^2$  and/or  $R^5$  would comprise as a moiety a heavy atom, such as a halide, more preferably chloride, bromide or iodide and most preferably bromide or iodide, to maximise population of the triplet state of the compound due to the "heavy atom effect". The heavy atom effect results in more efficient population of the triplet excited state of a photosensitizing compound. This can result, in turn, in a more efficient generation of singlet oxygen. Alternatively, if  $R^2$  and/or  $R^5$  is an alkyl, cyclic or heterocyclic moiety, it may be substituted with one or more heavy atoms, for example, a halide, more preferably chloride, bromide or iodide and most preferably bromide or iodide.

Alternatively,  $R^2$  and/or  $R^5$  would comprise a moiety or include as a substituent a water-solubilizing group to enhance the solubility of compounds of the present invention in aqueous solution. Suitable water-solubilizing groups include a moiety derived from sulfonic acids ( $-\text{SO}_3\text{H}$ ), alcohols ( $-\text{OH}$ ), carboxylic acids ( $-\text{COOH}$ ), amines ( $-\text{NR}_2$ ,  $-\text{N}^+\text{R}_3$ ), amides ( $-\text{NHCOR}$ ,  $-\text{CONHR}$ ), tetrazoles ( $-\text{CN}_4\text{R}$ ), sulphonamides ( $-\text{NHSO}_2\text{R}$ ,  $-\text{SO}_2\text{NHR}$ ) in which R is hydrogen or a substituted or unsubstituted, straight or branched chain alkyl moiety (which has from 1 to 25, preferably 1-10 and more preferably 1-4, carbon atoms).

$R^3$  and/or  $R^4$  preferably comprise, independently, a cyclic, preferably an aryl, moiety or a heterocyclic, preferably a heteroaryl, moiety. These moieties may be substituted or unsubstituted. Aryl moieties are more preferred, with monocyclic aryl moieties, and in particular phenyl, being most preferred.



R<sup>3</sup> and/or R<sup>4</sup> may be substituted with one or more heavy atoms, for example, a halide.

- Advantageously R<sup>3</sup> and/or R<sup>4</sup> comprise a moiety, or include substituents, that would maximise localisation of the compound in the tissue to be treated and optimise lipophilicity of the compound. Suitable substituents for alkyl; cyclic, preferably aryl; or heterocyclic, preferably heteroaryl, moieties to optimise lipophilicity include, but are not limited to, moieties derived from carboxylic acids (-COOH), sulfonic acids (-SO<sub>3</sub>H), phenols (-OH), alcohols (-OH), amines (-NR<sub>2</sub>, -N<sup>+</sup>R<sub>3</sub>), amides (-NHCOR, -CONHR), tetrazoles (-CN<sub>4</sub>R), sulphonamides (-NHSO<sub>2</sub>R, -SO<sub>2</sub>NHR) and esters (-COOR), in which R is a substituted or unsubstituted, straight or branched chain alkyl moiety (which has from 1 to 25, preferably 1-10 and more preferably 1-4, carbon atoms)
- Suitable substituents for alkyl, cyclic or heterocyclic moieties or, alternatively, suitable alkyl, cyclic or heterocyclic moiety to improve localisation within the tissue to be treated, for example, the cancerous region, include, but are not limited to, certain carbohydrates including β-D-galactose known to play a role in tumour cell recognition (C. Kieda, & Monsigny, M. (1986). "Involvement of membrane sugar receptors and membrane glycoconjugates in the adhesion of 3LL subpopulations to cultured pulmonary cells." *Invasion Metastasis*, **6**, 347-366); certain tripeptide sequences including Arg-Gly-Asp and Asn-Gly-Arg known for their utility in targeting doxorubicin to new blood vessels within tumours (Barinaga, M. (1998) "Peptide-guided cancer drugs show promise in mice" *Science*, **279**, 323-324 and Arap, W., Pasqualini, R., and Ruoslahti, E. (1998) "Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model" *Science*, **279**, 377-380); and certain steroids including 17β-oestradiol which may increase targeting of oestrogen receptor-positive breast cancer cells (Ferguson, A.T., Lapidus, R.G., and Davidson, N.E. (1998) "The regulation of estrogen receptor expression and function in human breast cancer" *Cancer Treat. Res.*, **94**, 255-278).

Preferably, the compounds of the present invention have an extinction coefficient of greater than  $30,000 \text{ M}^{-1}\text{cm}^{-1}$ , more preferably greater than  $50,000 \text{ M}^{-1}\text{cm}^{-1}$ , even more preferably greater than  $70,000 \text{ M}^{-1}\text{cm}^{-1}$ , and a maximum absorbance at  
5 greater than 640nm, preferably greater than 650nm as measured in water : Cremophor solution (100:1 (v/v)). Advantageously, the compounds of the present invention are, *in vivo*, localised within the cytoplasm, but not the nucleus, of the cells of the target tissue/area to be treated.

10 The compounds of the present invention, as non-porphyrin sensitisers, are a good starting point as they are amenable to modification of the phenyl rings around the periphery of the molecule to optimise their therapeutic properties. As a result of their ease of synthesis and purification, arrays of compounds with systematic structural variation can be generated to optimise the desired chemical,  
15 photophysical and biological properties of the photosensitising compounds of the invention.

In a second aspect, the invention provides a pharmaceutical composition, comprising a compound of the first aspect of the present invention in association  
20 with a pharmaceutically acceptable diluent or carrier.

In a third aspect, the invention provides a method of treating a photosensitive target biological cell *in vivo* or *in vitro*, the method comprising contacting the target biological cell with an effective amount of a compound of the first aspect of  
25 the invention or with an effective amount of a pharmaceutical composition of the second aspect of the present invention and then subjecting the photosensitive target biological cell with light absorbed by the said photosensitive cell, for example light at a wavelength of greater than 570nm, preferably greater than 600nm, still more preferably greater than 650nm.

In a fourth aspect, the invention provides use of a compound of the first aspect of the invention, preferably in association with a pharmaceutically acceptable diluent or carrier, in the preparation of a medicament of use in the therapy *in vivo* or *in vitro* of a photosensitive target biological cell by irradiation.

5

In further aspects, the invention provides: a method of photodynamic therapy, comprising administering a compound of the first aspect of the invention, preferably in association with a pharmaceutically acceptable diluent or carrier; and the use of a compound of the first aspect of the invention, preferably in association with a pharmaceutically acceptable carrier or diluent, in the manufacture of a medicament for the treatment of tumours in association with light, preferably of a wavelength of greater than 570 nm, more preferably greater than 600 nm, still more preferably greater than 650 nm.

15 These compositions are useful for sensitising a target biological substrate, for example, a tumour cell or other target, for example, an abnormal cell to destruction by irradiation using visible or near-visible light.

20 Typical indications, known in the prior art, include destruction of tumour tissue in solid tumours; dissolution of plaques in blood vessels; treatment of topical conditions such as acne, athletes foot, warts, papilloma, psoriasis and treatment of biological products, such as blood for transfusion, for infectious agents.

25 The compositions are formulated in pharmaceutical compositions for administration to the human or animal subject or applied to an *in vitro* target. The compositions can be administered systemically, in particular by injection, or can be used topically.

30 Injection may be intravenous, subcutaneous, intramuscular or intraperitoneal. Injectable compositions can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid

prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain minor amounts of non-toxic auxillary substances such as wetting or emulsifying agents, pH buffers and the like.

5

Systemic administration can be achieved, alternatively, through implantation of a slow release or a sustained release system, for example by suppository or orally, if so formulated.

- 10 If the treatment is to be localised, such as for the treatment of superficial tumours or skin disorders, the compositions can be topically administered using standard topical compositions such as lotions, suspensions or pastes.

- The invention will now be further described, with reference to the following non-limiting examples:-
- 15

### Assay Methods

#### **Cell culture**

- 20 MRC5 and HeLa cell cultures were maintained in MEM (minimum essential medium) containing 10% (v/v) foetal calf serum (FCS), 1% (v/v) non-essential amino acids, 2 µg/ml Fungizone (Trade Mark) (amphotericin B), 50 µg/ml penicillin, 50 µg/ml streptomycin, 20 mM HEPES and 1% (v/v) L-Glutamine. Cells were passaged at least twice before use in the cytotoxicity assays.

25

#### **Cytotoxicity studies (Haematoporphyrin)**

- Stock haematoporphyrin water-dimethyl sulphoxide (DMSO) (100:1 (v/v)) solutions were diluted with MEM containing 10% FCS. Cells were seeded at 5,000 cells/well in 96-well plates and incubated for 24 hr at 37°C. The 24 hour incubation period was chosen having regard to the slower uptake of haematoporphyrin in the absence of Cremophor, when compared to the
- 30

compounds of the present invention in the presence of Cremophor (see below). Cells were then incubated with haematoporphyrin in the dark for 24 hr at 37°C. The haematoporphyrin laden culture medium was then removed by filtration, the cells were washed with PBS and fresh culture medium was added to each well. A  
5 500W light source, passed through a red glass and water filter barrier, (to ensure cells are irradiated with light of wavelength greater than 570nm) was used to irradiate the plates for both 15 and 30 min. Following irradiation, the cells were incubated for a further 48 hr at 37°C before being assessed for cell survival. The dark toxicity of haematoporphyrin was also assessed in each experiment to show  
10 that any measured cell death was due to light illumination itself.

#### **Cytotoxicity studies (Compounds of the Invention)**

Stock **2a** or **2b** water-Cremophor EL (Trade Mark – CAS 61791-12-6) (1:250 (v/v)) solutions were diluted with MEM containing 10% FCS. Cells were seeded  
15 at 5,000 cells/well in 96-well plates and incubated for 24 hr at 37°C. Cells were then incubated with **2a** or **2b** in the dark for 3 hr at 37°C. The 3 hr incubation period was chosen to reflect the faster cellular uptake of **2a** or **2b** in the presence of Cremophor, when compared to the cellular uptake of haematoporphyrins in the absence of Cremophor. The **2a** or **2b** laden culture medium was then removed by  
20 filtration, the cells were washed with PBS and fresh culture medium was added to each well. The plates were then irradiated using a 500W light source passed through a red glass and water filter barrier, (thereby ensuring cells are irradiated with light of wavelength greater than 570nm) for both 15 and 30 min.  
  
25 Following irradiation, the cells were incubated for a further 48 hr at 37°C before being assessed for cell survival. The dark toxicity of compounds of the present invention was also assessed in each experiment.

#### **Measurement of cell viability**

30 Cell viability was estimated using the standard MTT (microtiter tetrazolium) assay. This assay measures mitochondrial dehydrogenase activity and is based on

the reduction of a soluble yellow tetrazolium salt to a blue, insoluble MTT formazan product by this enzyme. The subsequent colour change produced by viable cells was quantified using a plate reader (VICTOR<sup>2</sup> 1420 multilabel HTS counter, Wallac). As mentioned above under the headings "Cytotoxicity", the  
5 cells are incubated at 37°C for 48 hours, following which the MTT solution was added to the cells at a final concentration of 0.5 mg/ml and incubated for 3 hr at 37°C. The MTT solution was then removed by filtration and 100 µl DMSO was added to each well in order to lyse the cells and release the formazan dye. The plates were read 1 hr later at 540 nm.

10

#### **Preparation of cells for microscopy**

Cells were seeded at a density of 30,000 cells/well in chamber slides and allowed to adhere for 24 hr. **2a** ( $10^{-5}$  M) was then added to each chamber and incubated at 37°C in the dark for either 1 hr or 3 hrs at 37°C - a one hour incubation was used  
15 for Figs. 4 and 5. The medium was removed and the cells were washed 4 times with drug-free (**2a** free) medium. The cells were then fixed with 3.7% (v/v) formaldehyde for 15 min at 37°C. The fixative solution was removed and the cells were washed twice with sterile PBS. DAPI (4', 6-diamidino-2-phenylindole) nuclear stain (1/1000) was then incubated with the cells for 10 min at 37°C,  
20 following which the cells were washed twice with PBS. Cells were then mounted in Vectashield (Trade Mark) mounting medium.

#### **Fluorescence microscopy**

The cells were viewed using an Axio Zeiss fluorescent microscope. The cells  
25 were examined at the different time points using two different filters. The rhodamine filter, which is specific for the wavelength region in which **2a** fluoresces, was used to visualise **2a**. The DAPI filter was employed to examine the nuclei of the cells.

**Confocal microscopy**

The cells were viewed using a Leica TCSSL Confocal Laser Scanning microscope (the LSM510 META Confocal Microscope was used to visualise DAPI and 2a simultaneously).

5

**Fluorescence microscopy: Uptake study**

Cells were seeded at a density of 30,000 cells/well in chamber slides and allowed to adhere for 24 hr. A compound of the present invention ( $10^{-5}$  M) was then added to each chamber and incubated at 37°C in the dark over time, for example, 15 min, 30 min, 1 hr, 3 hr and 6 hr. The medium was removed after the specified time and the cells were washed 4 times with medium free of that compound of the invention. The cells were then fixed with 3.7% (v/v) formaldehyde for 15 min at 37°C. The fixative solution was removed and the cells were washed twice with sterile PBS. DAPI nuclear stain (1/1000) was then incubated with the cells for 10 min at 37°C, following which the cells were washed twice with PBS. Cells were then mounted in Vectashield (Trade Mark) mounting medium.

15

**Fluorescence microscopy: Efflux study**

Cells were seeded at a density of 30,000 cells/well in chamber slides and allowed to adhere for 24 hr. A compound of the present invention ( $10^{-5}$  M) was then added to each chamber and incubated at 37°C in the dark for 3 hrs. The same procedure was used as for the uptake studies, but for the efflux studies, the cells were treated with fixative solution at specified times after removal of the drug, for example, 5 min, 15 min, 30 min, 1 hr, 2 hr, 3 hr, 6hr and 24hr. The slides were then viewed using an Axio Zeiss fluorescent microscope. The cells were examined at the different time points using two different filters. The rhodamine filter was used to visualise the compound of the present invention. Due to its inherent fluorescent properties, the compounds of the present invention fluoresce red when viewed under the rhodamine filter. The DAPI filter was employed to look at the nuclei of the cells, which fluoresced blue due to treatment of DAPI, a nuclear stain.

25

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LabWorks (Bioimaging Systems) was used to calculate the average fluorescence intensity per cell for each of the different time points used in the uptake and efflux studies for the compounds of the present invention. For each time point, 5 fields of view were examined and each uptake/efflux experiment was performed in duplicate. The DAPI filter was used to accurately count the number of cells in each field of view and the rhodamine filter was used to quantify the fluorescence of the compounds of the present invention.

#### Data analysis

Prism (Trade Mark) (Bioimaging Systems) was used to graph the data obtained from the MTT assays and the uptake/efflux experiments. This programme allows non-linear regression analysis and the generation of sigmoidal dose response curves. Prism (Trade Mark) also automatically calculates EC<sub>50</sub> values.

#### Example 1

Referring to the accompanying reaction scheme, synthesis of **1** was repeated using the reported three step literature procedure of Rogers (1943). In order to make the chromophore more rigid, i.e., more structurally constrained and to limit radiationless transitions, so it would have the potential to act as a PDT agent, we converted it into its BF<sub>2</sub> chelate **2** (72-83% yield) by reaction at room temperature for 16 hours with boron trifluoride diethyl etherate, diisopropylamine (DIEA) in CH<sub>2</sub>Cl<sub>2</sub>. As the introduction of a heavy atom into a chromophore is generally accepted to facilitate enhancement of triplet state population (a requirement for singlet oxygen generation), we brominated at room temperature for 2 hours the free  $\beta$ -position of both pyrrole rings of **1** with molecular bromine in toluene or benzene giving **3** in high yields (85-90%). Conversion of **3** into its BF<sub>2</sub> chelate **4** was readily achieved using the same conditions as for **1** (see top part of Reaction Scheme – Figure 1)(71-78%). It will be appreciated that other compounds of the present invention can be similarly prepared, by use of the appropriately substituted azadipyrromethene, in place of compound **1**.



Both **2** and **4** are metallic brown solids and have good solubility in organic solvents such as chloroform, toluene or THF (tetrahydrofuran) and were fully characterised by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and HRMS (high resolution mass spectroscopy).

5

Compound **2a** denotes Compound **2** of the Reaction Scheme, where Ar is Phenyl, whilst Compound **2b** denotes Compound **2** of the Reaction Scheme where Ar is paramethoxyphenyl. Similarly, Compounds **4a** and **4b** denote Ar as phenyl or as paramethoxyphenyl, respectively.

10

Compound **2a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 7.03 (2H, s), 7.40-7.53 (12H, m), 8.0-8.1 (8H, m).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 119.3, 128.8, 128.8, 129.6, 129.7, 129.8, 129.9, 131.1, 131.8, 132.5, 143.6.

15 EI-HRMS: 497.1868.

Compound **2b**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 3.85 (6H, s), 7.02 (6H, m), 7.45 (6H, m) 8.06 (8H, m).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 55.66, 114.51, 118.91, 124.42, 128.78, 129.45, 129.53,

20 131.83, 131.89, 131.95, 132.76, 143.40, 162.20.

EI-HRMS: 557.2085.

Compound **4a**:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 7.41-7.50 (12H, m), 7.70-7.73 (4H, m), 7.84-7.89 (4H, m)

25  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 109.8, 126.9, 127.0, 128.4, 128.6, 129.3, 129.5, 129.7, 129.8, 141.9, 143.3, 157.5.

EI-HRMS: 653.0076.

Compound **4b**:

30  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 3.85 (6H, s), 6.9 (4H, d), 7.40-7.46 (6H, m), 7.75 (4H, d), 7.84-7.87 (4H, m).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 55.5, 110.2, 113.8, 122.0, 128.2, 129.7, 130.9, 131.0, 132.7, 142.2, 144.0, 157.1, 161.7.

EI-HRMS: 713.0275.

## 5 **Example 2**

A study of the spectroscopic properties of **2a** and **4a** in chloroform demonstrated that they have a relatively sharp absorption band of 650 - 660 nm of high molar extinction coefficients  $\sim 80,000$ , with a full width at half maximum (fwhm) of  $\sim$  50 nm. Introduction of an electron donating methoxy group onto the phenyl rings adjacent to the pyrrole nitrogen resulted in an increase in extinction coefficient and a significant bathochromic shift of the absorption bands for **2b** and **4b** at 688 and 679 nm, respectively (Table 1, Fig 2). The absorption bands of each photosensitiser are relatively insensitive to solvent changes with solutions in water/Cremophor resulting in a further bathochromic shift of  $\sim 10$  nm (Table 1). Excitation of chloroform solutions of the **2a** and **4a** at 635 nm gave a fluorescence band at 672 and 673 nm respectively (Fig. 2, Table 2). The fluorescence quantum yield of **2a** was 0.34 and, as would be expected, is significantly reduced for **4a** (0.012) due to the internal heavy atom effect (Table 2). Similarly, **2b** had a fluorescence quantum yield of 0.36, while **4b** was 0.10. The reduction in fluorescence quantum yield in the series would imply more efficient population of the triplet excited state which would benefit singlet oxygen production. The ability of **2** and **4** to produce singlet oxygen would be a prerequisite to them being potential PDT agents.

**Table 1** Spectroscopic absorbance properties of **2** and **4**<sup>a</sup>

Compound	$\lambda_{\text{max}}^{\text{b}}$ (nm)	fwhm <sup>b</sup> (nm)	$\epsilon^{\text{b}}$ ( $\text{M}^{-1}\text{cm}^{-1}$ )	$\lambda_{\text{max}}^{\text{c}}$ (nm)	fwhm <sup>c</sup> (nm)
<b>2a</b>	650	49	79,000	658	53
<b>2b</b>	688	55	85,000	696	57
<b>4a</b>	650	47	79,000	651	57
<b>4b</b>	679	57	75,000	685	86

<sup>a</sup>Room temperature. <sup>b</sup> $\text{CHCl}_3$ . <sup>c</sup>Water/Cremophor. (100:1)

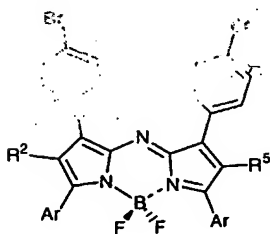
**Table 2** Extinction coefficients and fluorescence quantum yields ( $\Phi_f$ ) of **2** and **4**<sup>a</sup>

Compound	$\lambda_{em}$ (nm) <sup>b</sup>	$\Phi_f$ <sup>c</sup>	$\lambda_{em}$ (nm) <sup>d</sup>
<b>2a</b>	672	0.34	683
<b>2b</b>	715	0.36	727
<b>4a</b>	673	0.012	679
<b>4b</b>	714	0.10	719

<sup>a</sup>Room temperature. <sup>b</sup>CHCl<sub>3</sub>. <sup>c</sup>Relative to magnesium tetra-*tert*-butylphthalocyanine in CHCl<sub>3</sub> ( $\Phi_f = 0.84$ ).<sup>7</sup> <sup>d</sup>Water/Cremophor. (100:1)

Single crystal X-ray structure determination of **2b** demonstrated the conjugated  
 5 nature of the chromophore with similar bond lengths in both pyrrole rings and is  
 further confirmation of its molecular structure (Fig. 3).

### Example 3



10      a Ar = Ph; b Ar = 4-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>

### Compound 5

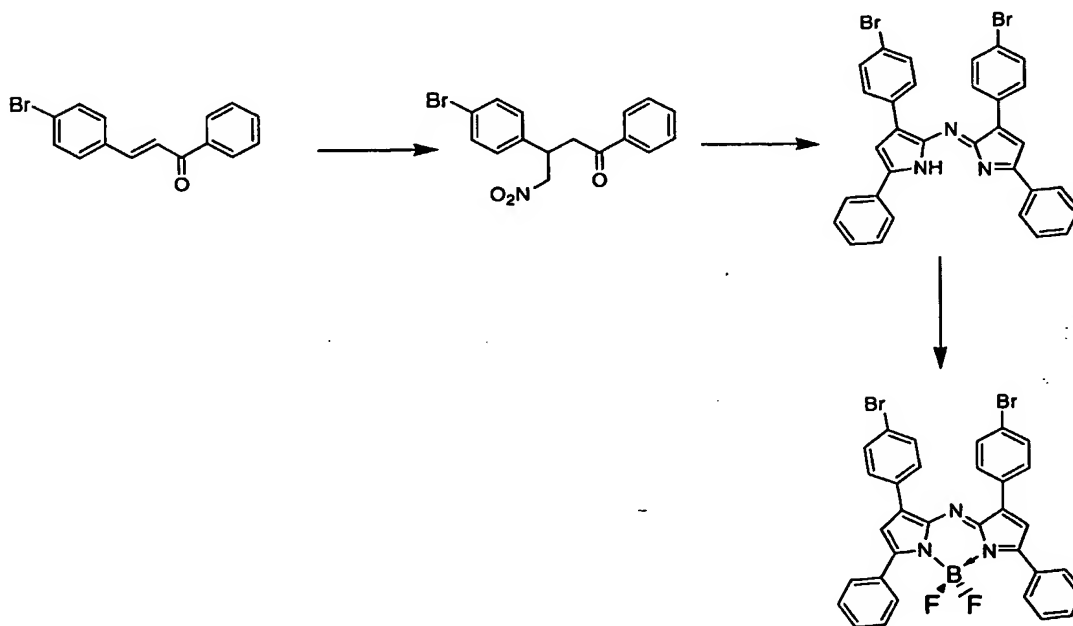
Compounds **5a** and **5b** denote compounds of the present invention, in which Ar is  
 phenyl or paramethoxyphenyl, respectively, and in which, in each case, the phenyl  
 15 group, on each ring, furthest from the pyrrole N is replaced with a  
 parabromophenyl group.

This positions a heavy atom (if required) on the phenyl rings allowing R<sup>1</sup> and R<sup>6</sup>  
 to incorporate an electron donating group e.g. para-OCH<sub>3</sub> (if required) and the  $\beta$ -

pyrrole substituent ( $R^2$ ,  $R^5$ ) could be either unsubstituted or contain a group which imparts another advantage such as enhanced water solubility (if required).

The method of synthesis is the same as described in the original papers referenced  
5 above and as outlined schematically below:-

The  $\text{BF}_3$  chelate is prepared according to the bottom part of the reaction scheme of Fig. 1.



10

Compound 5a:

$^1\text{H}$  NMR: 7.0 (2H, s), 7.47-7.61 (6H, m), 7.61 (4H, d), 7.90 (4H, d), 8.01-8.05  
(4H, m).  $\lambda_{\text{max}}$  ( $\text{CHCl}_3$ ): 658 nm. Extinction Coefficient  $74,000 \text{ M}^{-1}\text{cm}^{-1}$ .  $\lambda$

15  $\lambda_{\text{max}}$  ( $\text{H}_2\text{O}$ -Cremophor): 667 nm.  $\lambda_{\text{em}}$  ( $\text{CHCl}_3$ ): 680 nm.

EI-HRMS: 653.0081.

20

#### **Example 4**

Cancer cellular uptake of a photosensitiser is a prerequisite for it to act as a PDT agent. Delivery of our proposed PDT agents required formulation of the sensitisers in order to impart water solubility. Water / Cremophor solutions of **2a** ( $10^{-5}$  M) were added to HeLa cancer cell lines and incubated for 5, 15, 60 and 120 mins, washed with water and examined with fluorescent microscopy. Exploiting the inherent fluorescent properties of **2a**, efficient uptake and cytosolic localisation of **2a** was observed with maximum uptake after 300 minutes (Fig 4). Dual staining of the nucleus of the cells with 4',6-diamidino-2-phenylindole (DAPI) prior to treatment with **2a** gave good contrast imaging and confirmed localisation of **2a** primarily at the endoplasmic reticulum and not in the nucleus (Fig 5).

#### **Example 5**

Using the assay method set out above under the heading "Fluorescence Microscopy", the uptake of **2a** was examined for HeLa and MRC5 cancer cell lines and the data are illustrated in Figs. 6a and 6b, respectively. Thus, for the HeLa cells, a time-dependent uptake is illustrated with a maximum reached after 300 minutes whilst, for MRC5 cells, a time-dependent uptake is also shown, with a maximum reached after 200 minutes.

#### **Example 6**

Using the assay method set out above under the heading "Fluorescence Microscopy", the efflux of **2a** was examined from HeLa cells and the data are illustrated in Figure 7. The efflux is time-dependent, being complete in 1000 minutes.

These efflux data suggest that the compounds of the present invention are not retained in non-irradiated cells, post-treatment.

### **Example 7**

5

Preliminary light toxicity assays were carried out as follows:

HeLa cancer cells were exposed to **2a** in varying concentrations for 24 hours. **2a** laden medium was removed and replaced with fresh medium. Cells were  
10 irradiated at constant temperature of 37°C for 15 minutes with light from a 100 W or 500W halogen lamp passed through a red glass and water filter barrier, thereby ensuring cells are irradiated with light of more than 570nm. Irradiated cells were incubated for a further 24 hrs at 37°C. MTT cell viability assay was performed.

15 A typical dose response curve is shown in Fig 8. This demonstrates an EC-50 of  $1 \times 10^{-6} \text{M}$  using the 500W light source. The poor response for the 100 W light source demonstrates that varying the quantity of light activation has a direct effect on drug efficacy. The dark toxicity effect may be caused by the micellar delivery vehicle itself.

20

### **Example 8**

Light toxicity assays were also carried out as described above under "Assay methods" in HeLa and MRC5 cells for haematoporphyrin (Figs. 9 and 10), **2a**  
25 (Figs. 11 and 12) and **2b** (Figs. 13 and 14).

Prior art haematoporphyrin was assessed with HeLa cancer cells. Exposure to 0 J/cm<sup>2</sup> light (no light) gave an EC50 value of  $7.7 \times 10^{-5} \text{M}$ . Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source) or exposure to 16 J/cm<sup>2</sup> light (30 mins  
30 from a 500W light source) gave EC50 values of  $1.5 \times 10^{-5} \text{M}$  and of  $1.6 \times 10^{-5} \text{M}$ , respectively.

Prior art haematoporphyrin was also assessed with MRC5 cancer cells. Exposure to 0 J/cm<sup>2</sup> light (no light) gave an EC50 value of  $1.4 \times 10^{-3}$ M. Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source) or to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source) gave EC50 values of  $4.2 \times 10^{-5}$ M or  $2.9 \times 10^{-5}$ M, respectively.

Compound 2a was assessed with HeLa cells. Exposure to 0 J/cm<sup>2</sup> light (no light) gave an EC50 value of  $2.3 \times 10^{-5}$ M. Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source) or to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source) gave EC50 values of  $4.5 \times 10^{-6}$ M and  $1.3 \times 10^{-6}$ M, respectively.

Compound 2a was also assessed with MRC5 cancer cells. Exposure to 0 J/cm<sup>2</sup> light (no light) gave an EC50 value of  $3.5 \times 10^{-6}$ M. Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source) gave an EC50 value of  $2.0 \times 10^{-6}$ M, whilst exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source) gave an EC50 value of  $1.1 \times 10^{-6}$ M.

Compound 2b was assessed with HeLa cancer cells. Exposure to 0 J/cm<sup>2</sup> light (no light) gave an EC50 value of  $6.2 \times 10^{-5}$ M. Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source) gave an EC50 value of  $5.3 \times 10^{-5}$ M. Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source) gave an EC50 value of  $2.5 \times 10^{-5}$ M.

Compound 2b was also assessed with MRC5 cancer cells. Exposure to 0 J/cm<sup>2</sup> light (no light) gave an un-measurable EC50 value. Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source) gave an EC50 value of  $2.2 \times 10^{-4}$ M. Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source) gave an EC50 value of  $2.2 \times 10^{-5}$ M.

The EC50 data shows that both **2a** and **2b** are acting as PDT agents. **2a** was a significant improvement than the prior art compound haematoporphyrin and **2b** was also improved in comparison to haematoporphyrin. Increased light doses from 8 J/cm<sup>2</sup> to 16 J/cm<sup>2</sup> give rise to more favourable EC-50 values for both **2a** and **2b** thereby demonstrating that these compounds are acting as PDT agents as their effectiveness is dependant not only on the concentration of compound administered to the cells but also the quantity of light energy delivered to the cells.

These toxicity data were confirmed by reference to Fig 16 in contrast to Fig 15.

10

Fig 15 illustrates a control experiment which is a fluorescence microscope image of F-actin stained HeLa cells (with rhodamine phalloidine, red colour) 24 hours after treatment with Compound **2a** and no exposure to light (0 min light dose). Fig 15 illustrates such F-Actin stained cells are live.

15

Fig 16 confirms cell death following treatment with Compound **2a** and 30 minute 500W light dose, using fluorescence microscopy. More specifically, Fig 16 is a fluorescence microscope image of F-actin stained HeLa cells (with rhodamine phalloidine, red colour) 24 hours after treatment with Compound **2a** and 30 minute light dose.

20

#### **Example 9 - Confocal data**

Referring to Figs. 17-19, there are illustrated both diffuse and punctuated cytoplasmic localisation of **2a**. The diffuse spread is suggestive of mitochondrial localisation (see centre of cell of Fig. 18). The punctuated spread is suggestive of localisation in organelles of size range 1-2µm (see Fig. 19). These could be lysosomes which have a typical size range of 0.2-2µm; peroxisomes which have a typical size range of 0.5-1.5µm; or endosomes which have a typical size range of 0.2-2µm. It will be appreciated that localisation of the photosensitising

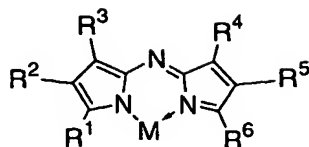
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compounds of the invention in different subcellular sites may impact on how effective the photodynamic therapy may be.

**Claims:**

1. A compound of the formula



or a salt, metal complex or hydrate or other solvate thereof, wherein:

M is a chelating agent;

R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> are independently selected from the group consisting of:

- 10 H; a substituted or unsubstituted, saturated or unsaturated, cyclic, moiety; a substituted or unsubstituted, saturated or unsaturated, heterocyclic moiety; or a substituted or unsubstituted, saturated or unsaturated, straight or branched chain alkyl or acyl moiety; and
- R<sup>2</sup> and R<sup>5</sup> may also be independently selected from a water-solubilizing group.

2. A compound as claimed in Claim 1, wherein M is selected from the group comprising: BX<sub>2</sub>, wherein each X is independently a halide; Zn; Al; Si; Mg; Lu; and Sn.

3. A compound as claimed in Claim 2, wherein M is BF<sub>2</sub>.

4. A compound as claimed in any one of Claims 1 to 3, wherein R<sup>1</sup> and R<sup>6</sup> are optionally substituted phenyl.

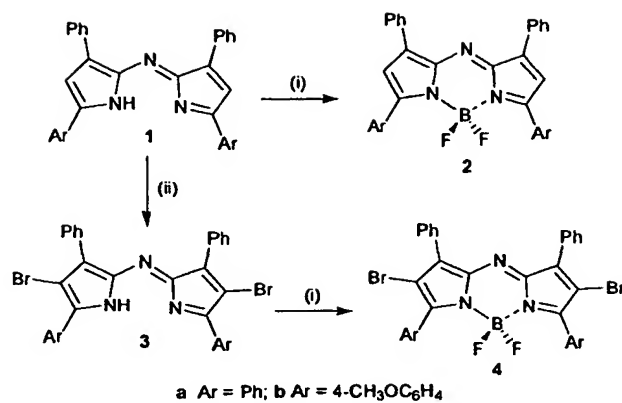
5. A compound as claimed in any one of Claims 1 to 4, wherein R<sup>1</sup> and R<sup>6</sup> contain an electron-donating substituent.

6. A compound as claimed in Claim 5, wherein the electron-donating substituent is alkoxy.

7. A compound as claimed in any one of Claims 1 to 6, wherein  $R^2$  and  $R^5$  are selected from chlorine, bromine and iodine.
- 5 8. A compound as claimed in any one of Claims 1 to 6, wherein  $R^2$  and  $R^5$  are selected from water-solubilizing groups.
9. A compound as claimed in any one of Claims 1 to 8, wherein  $R^3$  and  $R^4$  are optionally substituted phenyl.
- 10 10. A compound as claimed in Claim 9, wherein  $R^3$  and  $R^4$  are substituted by a halide selected from chlorine, bromine and iodine.
11. A pharmaceutical composition, comprising a compound as claimed in any  
15 one of Claims 1 to 10, in association with a pharmaceutically acceptable diluent or carrier.
12. A compound as claimed in any one of Claims 1 to 10 for use in a method of photodynamic therapy.
- 20 13. The use of a compound as claimed in any one of Claims 1 to 10 in the manufacture of a medicament of use in the therapy *in vivo* or *in vitro* of a photosensitive target biological cell by irradiation.
- 25 14. The use of a compound as claimed in any one of Claims 1 to 10 in the manufacture of a medicament of use in treatment of tumours in association with light.

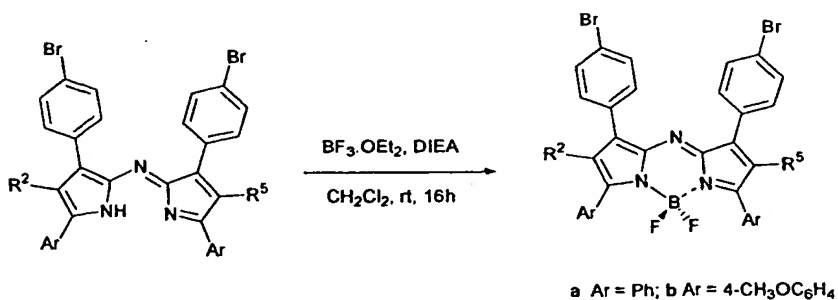
Fig. 1

## Reaction Scheme

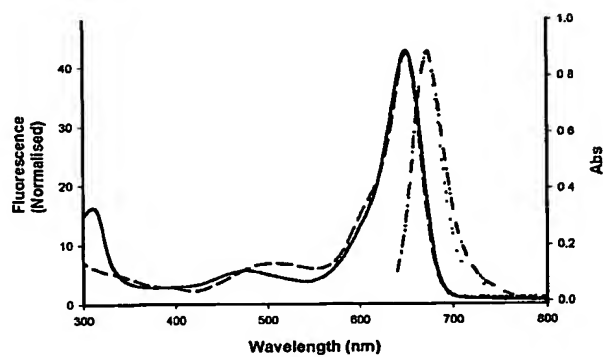


(i) BF<sub>3</sub>.OEt<sub>2</sub>, DIEA, CH<sub>2</sub>Cl<sub>2</sub>, rt, 16 h;

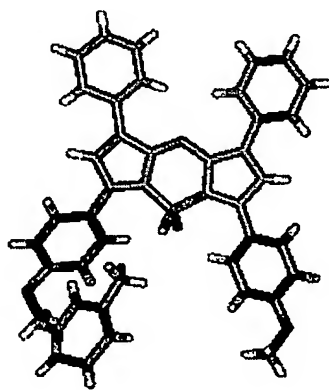
(ii) Br<sub>2</sub>, toluene, rt, 2 h.



**Fig. 2** Normalised absorption (—) and emission spectra (.....) of **2a** and electronic absorption (- - -) and fluorescence spectra ( - - - ) of **4a** in  $\text{CHCl}_3$  at room temperature.



**Fig. 3** X-Ray crystal structure of **2b**; crystallised from toluene/methanol bilayer (co-crystallised with molecule of toluene).



**Fig. 4** Cellular localisation of **2a** (light grey colour) in HeLa cancer cells visualised with fluorescent microscopy (darker grey area is the cell nucleus).



**Fig. 5** Cellular localisation of **2a** in HeLa cancer cells; nucleus is co-stained with DAPI (blue) and cytoplasmic localisation of **2a** (red).

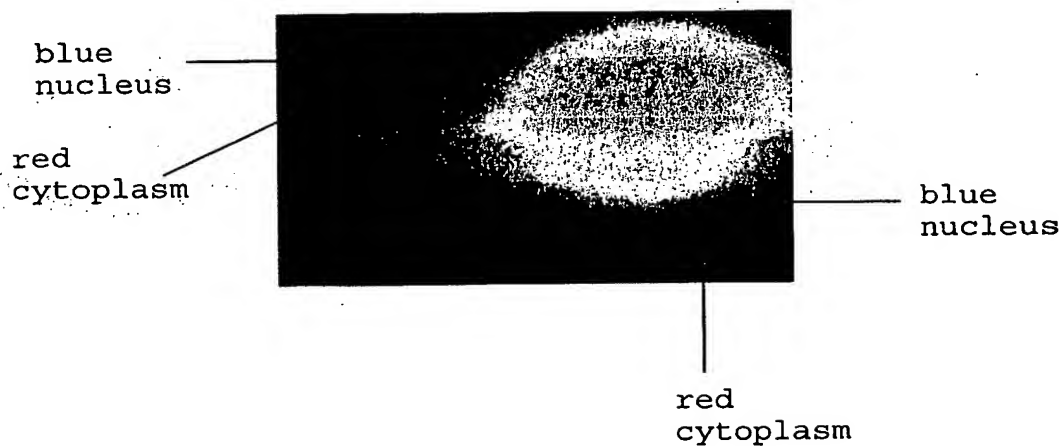


Fig. 6a:

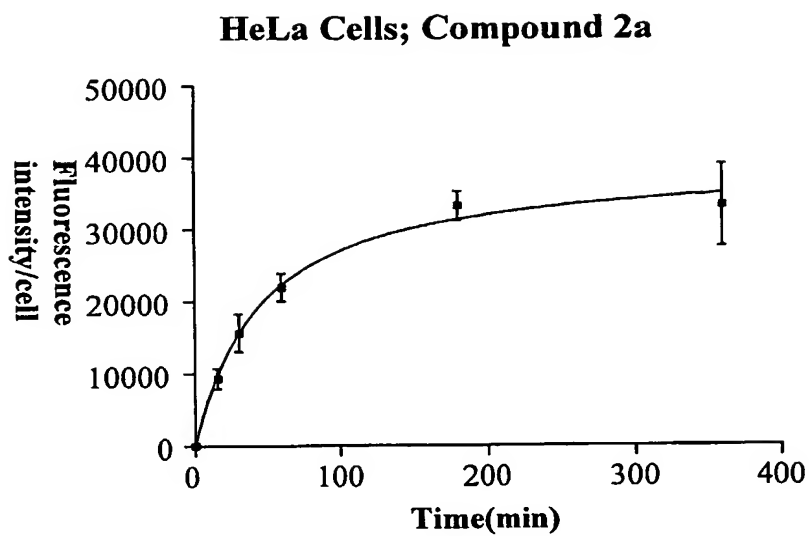


Fig. 6b:

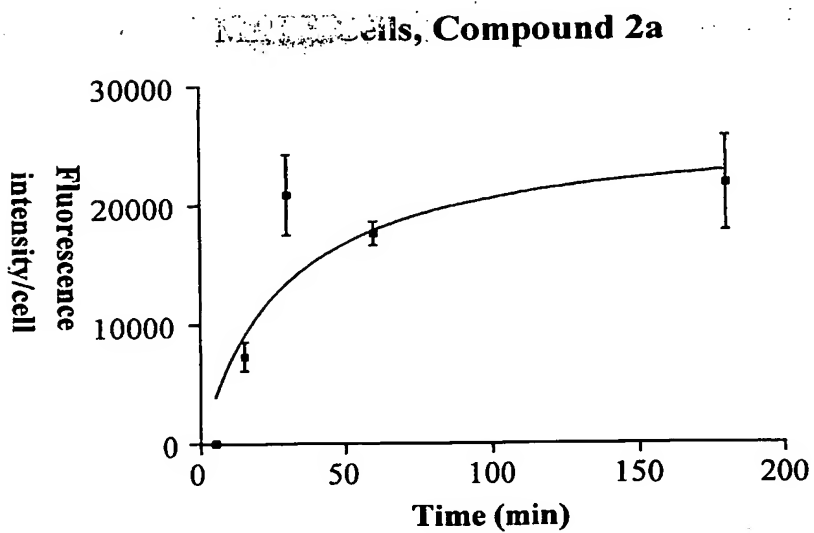


Fig. 7:

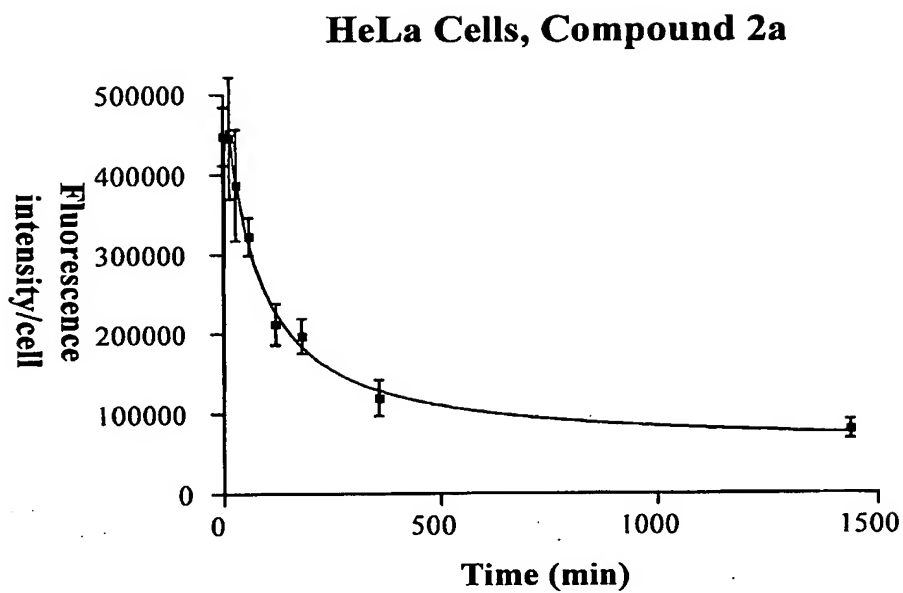


Fig 8. Dose Response Curve for 2a

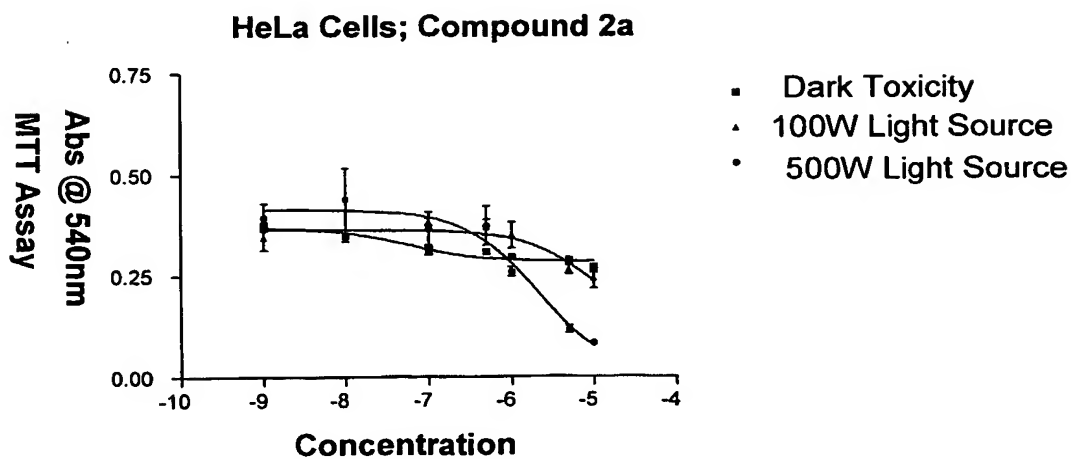
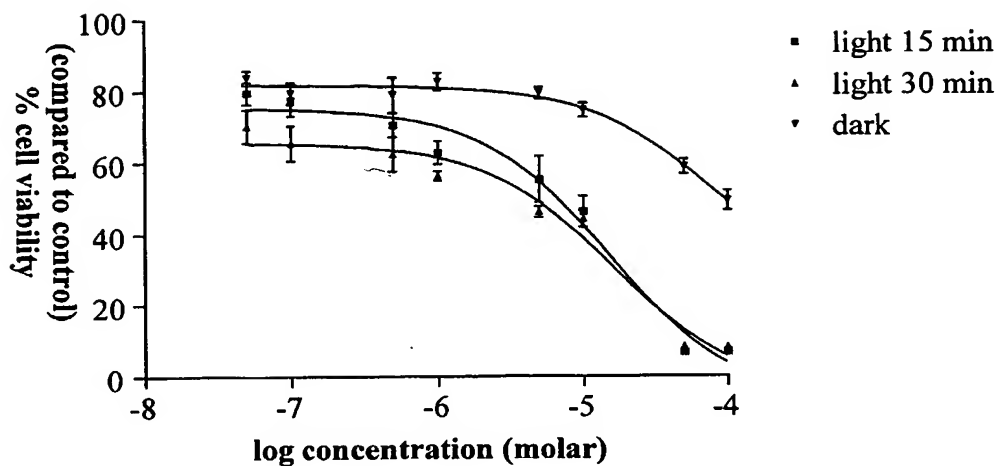


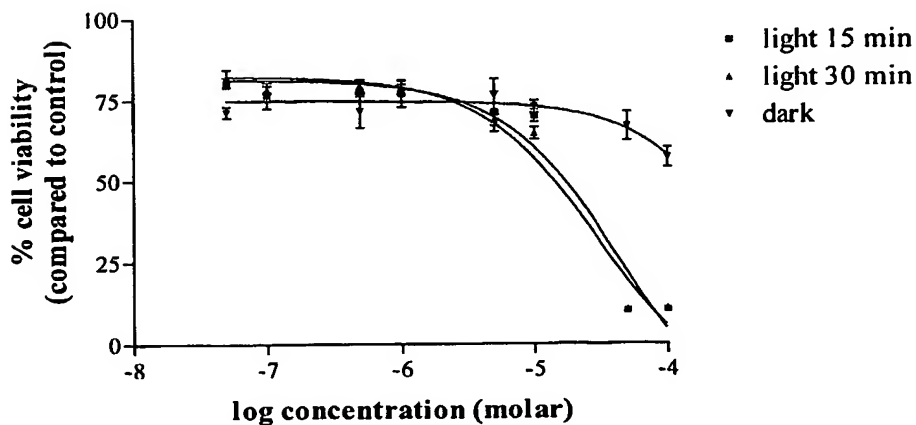


Fig. 9:

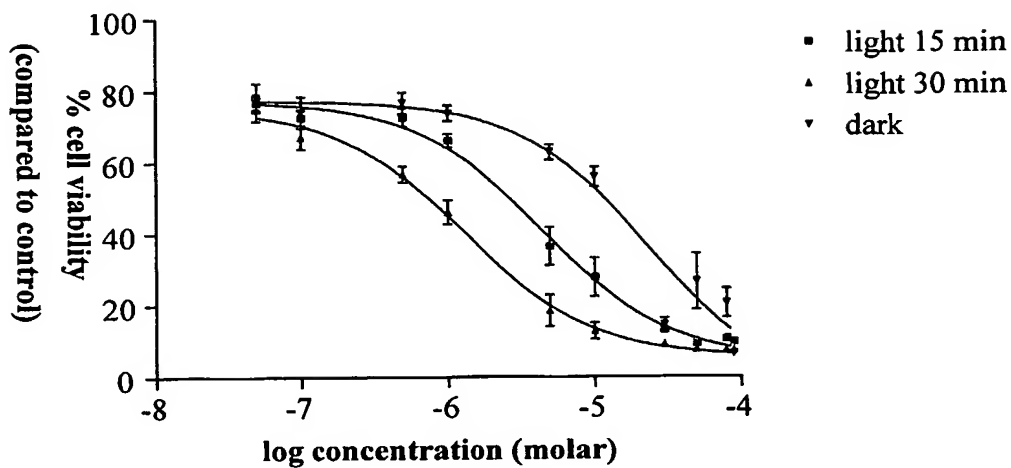
**HeLa: Haematoporphyrin**

- ▼ Exposure to 0 J/cm<sup>2</sup> light (no light)
- Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source)
- ▲ Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source)

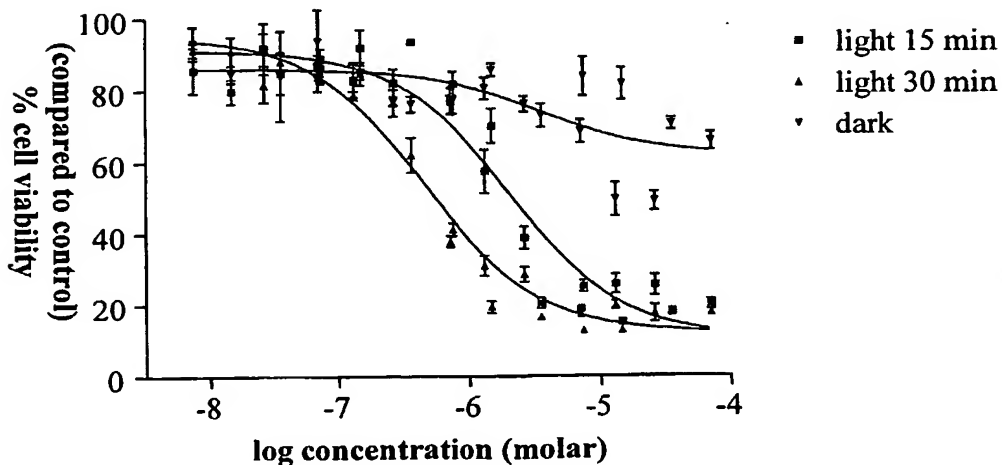
Fig. 10:

**MRC5: Haematoporphyrin (24 hr),  
500 W light, MTT after 48 hrs**

- ▼ Exposure to 0 J/cm<sup>2</sup> light (no light)
- Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source)
- ▲ Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source)

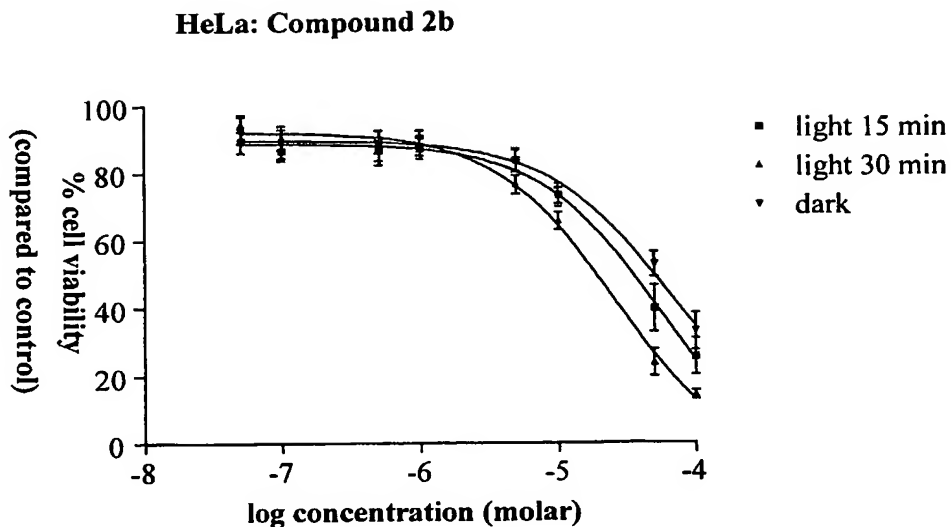
**Fig. 11****HeLa: Compound 2a**

- ▼ Exposure to 0 J/cm<sup>2</sup> light (no light)
- Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source)
- ▲ Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source)

**Fig. 12:****MRC5: Compound 2a**

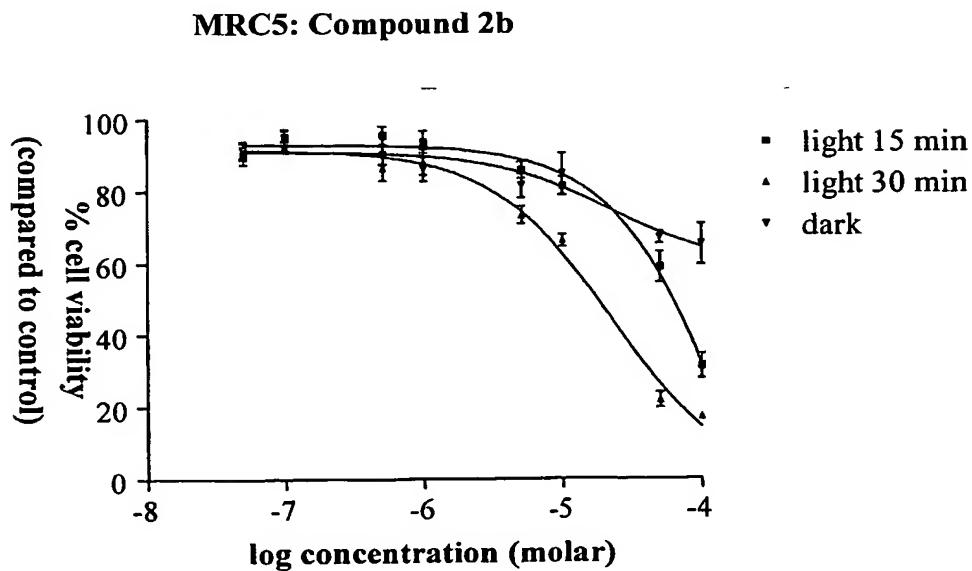
- ▼ Exposure to 0 J/cm<sup>2</sup> light (no light)
- Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source)
- ▲ Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source)

Fig. 13:



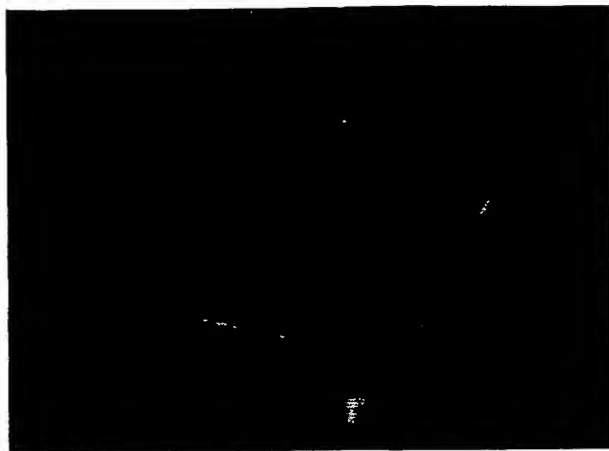
- ▾ Exposure to 0 J/cm<sup>2</sup> light (no light)
- Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source)
- ▴ Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source)

Fig. 14:



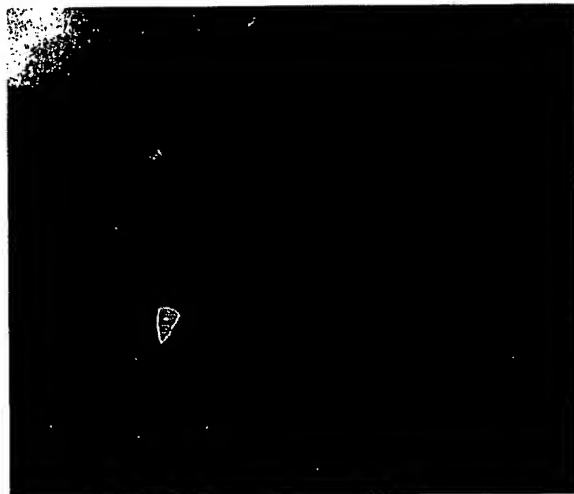
- ▾ Exposure to 0 J/cm<sup>2</sup> light (no light)
- Exposure to 8 J/cm<sup>2</sup> light (15 mins from a 500W light source)
- ▴ Exposure to 16 J/cm<sup>2</sup> light (30 mins from a 500W light source)

**Fig. 15:** Cell Toxicity following administration of 2a



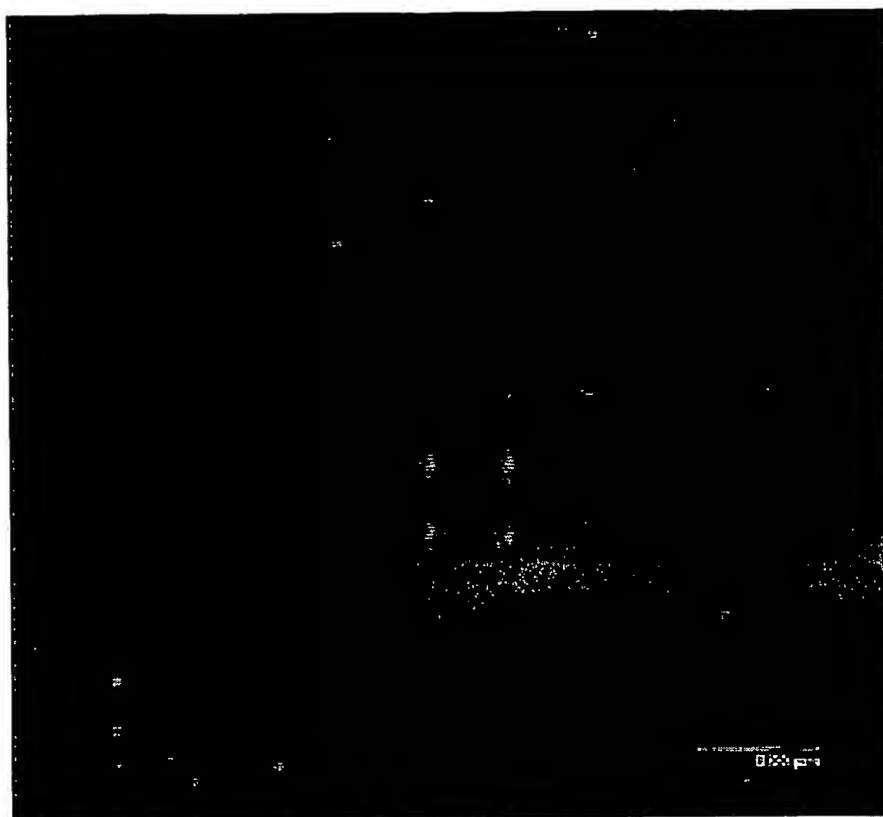
**Fig. 16:**

Cell Toxicity following administration of 2a and a light dose



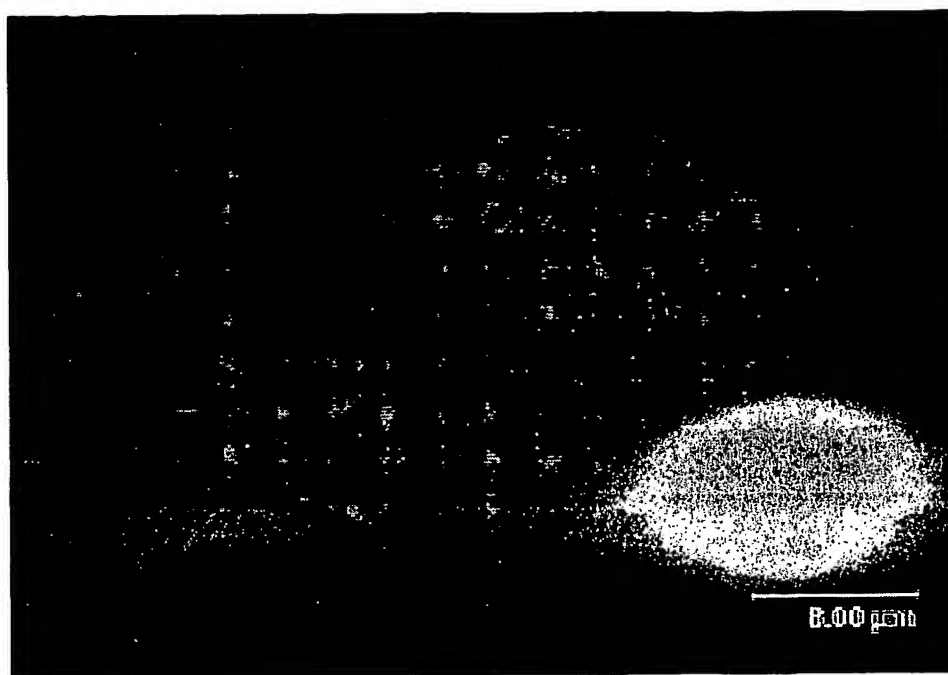
**Fig. 17:**

Confocal Microscope Image of Subcellular Localisation of Compound 2a (red)  
showing diffuse and punctuated subcellular localisation.



**Fig. 18:**

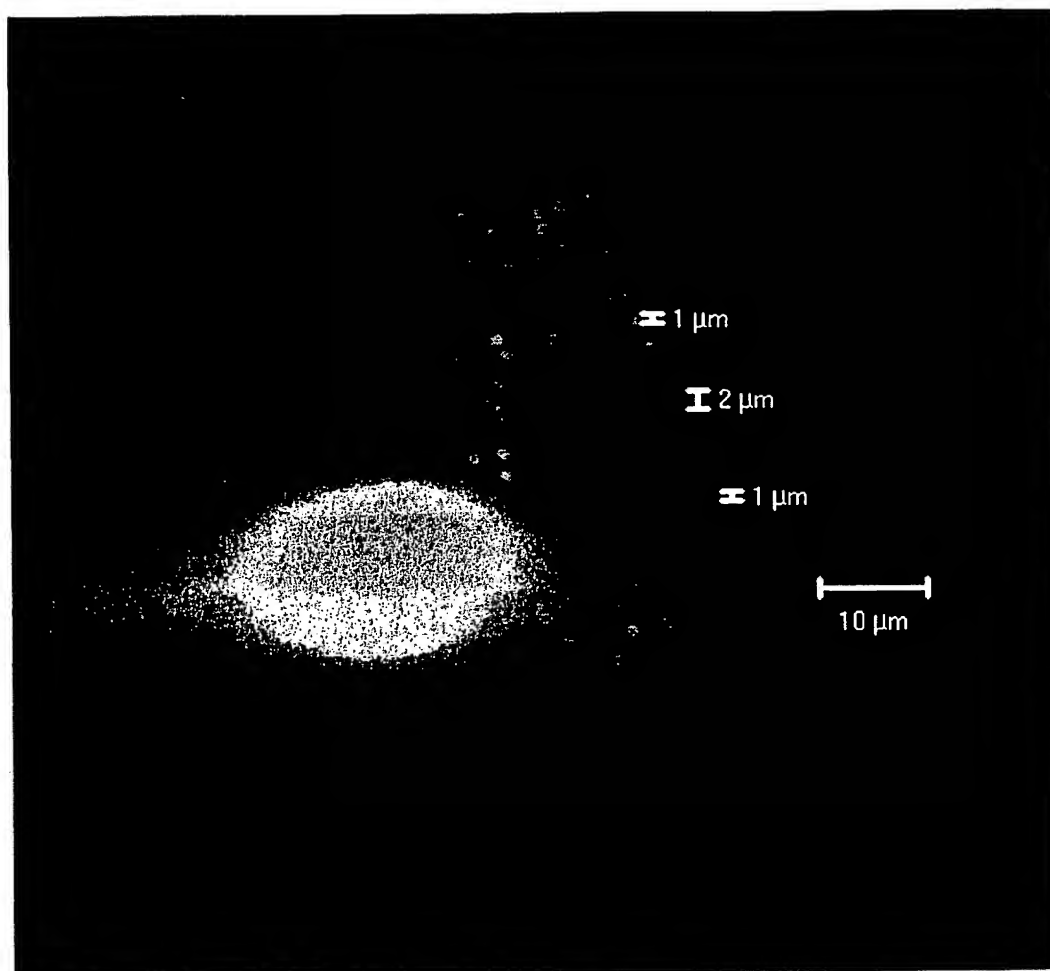
Confocal Microscope Image of Subcellular Localisation of Compound 2a (red) in HeLa Cells with a Sytox green nuclear co-stain.



**Fig. 19:**

Identification of punctuated localisation.

Confocal Microscope Image of Subcellular Localisation of Compound **2a** (red) in HeLa Cells with a DAPI (blue) nuclear co-stain.



## INTERNATIONAL SEARCH REPORT

Internat. Search No.

PCT/EP 03/03174

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07F5/02 A61N5/06 A61K31/69

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07F A61K A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

PAJ, EPO-Internal, CHEM ABS Data, WPI Data, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 1999, no. 09, 30 July 1999 (1999-07-30) & JP 11 092479 A (MITSUI CHEM INC;YAMAMOTO CHEM INC), 6 April 1999 (1999-04-06) See patent: formulas 2 and 3 at page 2 and the tables from page 7 to 10 abstract	1-10
X,Y	US 5,336 A (BOYER JOSEPH H ;MORGAN LEE R (US)) 4 April 1990 (1990-04-04) See compound 14 at page 14 page 3, line 53 -page 4, line 42 -/-	1-14

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

28 July 2003

Date of mailing of the international search report

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE CAPLUS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 1980 ABEYSEKERA, A. ET AL;: "Dicarbonylrhodium(I) complexes of polypyrrole macrocycles. Part 3. Synthesis and crystal structures of complexes of N-methylcorroles, N-methylporphyrins and acyclic polypyrroles" retrieved from STN, accession no. 1980:103437 Database accession no. 92:103437 XP002249181 See compound with RN: 72805-32-4 abstract</p> <p>---</p>	1,4,5,9
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P,X	<p>KILLORAN, J. ET AL: "Synthesis of BF<sub>2</sub> chelates of tetraarylazadipyrromethenes and evidence for their photodynamic therapeutic behaviour" CHEM. COMM., vol. 8, no. 17, - 21 August 2002 (2002-08-21) pages 1862-1863, XP002249180 see scheme 1 figures 2-4; tables 1,2</p> <p>-----</p>	1-14

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Internat - cation No

PCT/EP 03/03174

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